

WHAT IS CLAIMED IS:

5

1. A purified mammalian RapR6 protein.

2. The protein of claim 1 which comprises the amino acid sequence substantially as set forth in SEQ ID NO:3 or 11.

10

3. A purified protein encoded by a nucleic acid capable of hybridizing to a DNA having a sequence consisting of the coding region of SEQ ID NO:2 or 10.

4. A purified derivative or analog of the protein of claim 1, which displays one or more functional activities of a mammalian RapR6 protein.

15

5. The derivative or analog of claim 4 which is capable of binding to an antibody directed against a mammalian RapR6 protein.

6. A purified fragment of a mammalian RapR6 protein.

20

7. The fragment of claim 6, wherein said fragment comprises a WD40 domain of a mammalian RapR6 protein.

8. The fragment of claim 7, wherein said mammalian RapR6 protein is a human RapR6 protein, and wherein said fragment comprises amino acids 24-63 of said RapR6 protein.

25

9. The fragment of claim 7, wherein said mammalian RapR6 protein is a human RapR6 protein, and wherein said fragment comprises amino acids 66-105 of said RapR6 protein.

30

10. The fragment of claim 7, wherein said mammalian RapR6 protein is a human RapR6 protein, and wherein said fragment comprises amino acids 108-154 of said RapR6 protein.

35

11. The fragment of claim 7, wherein said mammalian RapR6 protein is a human RapR6 protein, and wherein said fragment comprises amino acids 203-244 of said RapR6 protein.

12. The fragment of claim 7, wherein said mammalian RapR6 protein is a human RapR6 protein, and wherein said fragment comprises amino acids 247-287 of said RapR6 protein.

13. The fragment of claim 6, wherein said fragment comprises a transmembrane  
5 domain of a mammalian RapR6 protein.

14. The fragment of claim 13, wherein said mammalian RapR6 protein is a human  
RapR6 protein, and wherein said fragment comprises amino acids 1-21 of said RapR6  
protein.

10 15. The fragment of claim 13, wherein said mammalian RapR6 protein is a human  
RapR6 protein, and wherein said fragment comprises amino acids 210-232 of said RapR6  
protein.

16. The fragment of claim 6, wherein said fragment does not comprise a WD40  
15 domain or a transmembrane domain of a mammalian RapR6 protein.

17. The fragment of claim 16, wherein said mammalian RapR6 protein is a human  
RapR6 protein, and wherein said fragment comprises amino acids 155-202 of said RapR6  
protein.

20 18. A molecule comprising the fragment of any one of claims 6-17.

19. A protein comprising an amino acid sequence that has at least 60% identity to a  
domain of a mammalian RapR6 protein, in which the percentage identity is determined over  
an amino acid sequence of identical size to the domain.

25 20. A protein comprising an amino acid sequence that has at least 90% identity to a  
domain of a mammalian RapR6 protein, in which the percentage identity is determined over  
an amino acid sequence of identical size to the domain.

21. A polypeptide comprising a fragment of a mammalian RapR6 protein consisting  
30 of at least 6 amino acids fused via a covalent bond to an amino acid sequence of a second  
peptide, wherein said second peptide is not comprised in a mammalian RapR6 protein.

22. The polypeptide of claim 21, wherein the fragment of the mammalian RapR6  
protein is a fragment capable of binding to an anti-RapR6 protein antibody.

35 23. The polypeptide of claim 22, wherein the fragment capable of binding to an anti-  
RapR6 protein antibody further lacks one or more domains of the RapR6 protein.

24. An antibody which is capable of binding to a mammalian RapR6 protein.

25. The antibody of claim 24 which is a monoclonal antibody.
- 5 26. A molecule comprising a fragment of the antibody of claim 26, wherein said fragment is capable of binding to a RapR6 protein.
27. An isolated nucleic acid comprising a nucleotide sequence encoding a mammalian RapR6 protein as set forth in SEQ ID NO:2 or 10.
- 10 28. The nucleic acid of claim 27 which is DNA.
29. An isolated nucleic acid comprising a nucleotide sequence complementary to the nucleotide sequence of claim 27.
- 15 30. An isolated nucleic acid hybridizable to the nucleic acid of claim 29.
31. An isolated nucleic acid comprising a fragment of a mammalian RapR6 gene consisting of at least 8 nucleotides.
- 20 32. An isolated nucleic acid comprising a fragment of a mammalian RapR6 gene comprising any one of exons 1-6 of a mammalian RapR6 gene.
33. An isolated nucleic acid comprising a fragment of a mammalian RapR6 gene comprising an intron, or a fragment thereof, of a mammalian RapR6 gene.
- 25 34. An isolated nucleic acid comprising a nucleotide sequence encoding a fragment of a mammalian RapR6 protein that displays one or more functional activities of the mammalian RapR6 protein.
35. An isolated nucleic acid comprising a nucleotide sequence encoding any one of the fragments of claims 6-17.
- 30 36. A recombinant cell containing the nucleic acid of claim 35.
37. A method of producing a mammalian RapR6 protein comprising:
- 35 (a) growing a recombinant cell containing the nucleic acid of claim 36 such that the encoded fragment of said mammalian RapR6 protein is expressed by the cell; and
- (b) recovering said expressed fragment of said mammalian RapR6 protein.

38. The product of the process of claim 37.

5

39. A pharmaceutical composition comprising a therapeutically effective amount of a mammalian RapR6 protein and a pharmaceutically acceptable carrier.

10

40. A pharmaceutical composition comprising a therapeutically effective amount of an antibody capable of binding to a mammalian RapR6 protein and a pharmaceutically acceptable carrier.

15

41. A method for generating a genetically modified cell having altered sensitivity to rapamycin, said method comprising introducing into the genome of a cell of a selected cell type of an organism a knockout DNA construct, said knockout DNA construct comprising (i) a regulated promoter and (ii) a selection marker coding sequence under the control of said regulated promoter, wherein said regulated promoter, when activated, initiates RNA transcription to produce an RNA; wherein, when said regulated promoter is activated, said genetically modified cell is rapamycin resistant if cells of said selected cell type is rapamycin sensitive or is rapamycin sensitive if cells of said selected cell type is rapamycin resistant.

20

42. The method of claim 41, wherein said knockout DNA construct further comprises a rapid cloning element comprising a replication origin sequence comprising sequences for initiation of replication and segregation and a bacterial selection marker.

25

43. The method of claim 42, wherein said replication origin sequence is an Ori and said bacterial selection marker is a chloramphenicol resistance gene.

30

44. The method of claim 41, wherein said method further comprising activating said regulated promoter and identifying said genetically modified cell by a method comprising identifying a change in rapamycin resistance in said genetically modified cell.

35

45. The method of claim 42, further comprising cloning a fragment of genomic sequence by a method comprising: (a) obtaining a nucleotide sequence comprising said rapid cloning element and said fragment of genomic sequence; (b) circularizing said nucleotide sequence to generate a circular plasmid; and (c) transforming a suitable host cell using said circular plasmid.

46. The method of claim 44, further comprising determining the sequence of said fragment of genomic sequence by a method comprising sequencing said circular plasmid.

47. The method claim 45, further comprising determining the location of said  
5 fragment of genomic sequence in said genome of said cell by a method comprising  
comparing said sequences with the genomic sequence of said selected cell type.

48. The method of claim 41, wherein said method further comprising, prior to said  
step of introducing said knockout DNA construct, introducing into the genome of cells of  
10 said selected cell type a DNA construct encoding a transactivator, said DNA construct  
comprising (i) a promoter and (ii) a nucleotide sequence encoding a transactivator, said  
nucleotide sequence being under the control of said promoter, wherein said regulated  
promoter is activated by said transactivator, and wherein said genetically modified cell is  
generated by introducing said knockout DNA construct into a cell comprising said DNA  
15 construct encoding said transactivator.

49. The method of claim 48, wherein said regulated promoter is a tetracycline  
regulated promoter, and wherein said transactivator activates said regulated promoter in the  
absence of tetracycline.

50. The method of claim 49, wherein said knockout DNA construct further  
20 comprises a rapid cloning element comprising a replication origin sequence comprising  
sequences for initiation of replication and segregation and a bacterial selection marker.

51. The method of claim 50, wherein said replication origin sequence is an Ori and  
25 said bacterial selection marker is a chloramphenicol resistance gene.

52. The method of claim 51, wherein said method further comprising identifying  
said genetically modified cell by a method comprising identifying a change in rapamycin  
resistance in said genetically modified cell.

53. The method of claim 51, further comprising cloning a fragment of genomic  
30 sequence by a method comprising: (a) obtaining a nucleotide sequence comprising said  
rapid cloning element and said fragment of genomic sequence; (b) circularizing said  
nucleotide sequence to generate a circular plasmid; and (c) transforming a suitable host cell  
using said circular plasmid.

54. The method of claim 53, further comprising determining the sequence of said  
35 fragment of genomic sequence by a method comprising sequencing said circular plasmid.

55. The method of claim 54, further comprising determining the location of said  
5 fragment of genomic sequence in said genome of said cell by a method comprising  
comparing said sequences with the genomic sequence of said selected cell type.

56. The method of any one of claims 41-55, wherein said selected cell type is a  
rapamycin sensitive cell type.

10 57. The method of claim 56, wherein said organism is a human.

58. The method of claim 56, wherein said organism is a mouse.

59. The method of claim 58, wherein said selected cell type is the murine  
15 neuroblastoma N2a cell line.

60. The method of claim 56, wherein said knockout DNA construct is integrated at a  
location in a RapR6 gene.

61. The method of any one of claims 41-55, wherein said selected cell type is a  
20 rapamycin resistant cell type.

62. The method of claim 61, wherein said organism is a human.

63. The method of claim 61, wherein said organism is a mouse.

64. A method for treating a mammal having a cancer, said cancer being caused by  
25 defective regulation of a RapR6 gene and/or defective activity of a protein encoded by said  
RapR6 gene, said method comprising administering to said mammal a therapeutically  
sufficient amount of an agent, said agent regulating the expression of said RapR6 gene  
and/or activity of said protein encoded by said RapR6 gene.

30 65. The method of claim 64, wherein said cancer is caused by a mutation in said  
RapR6 gene, and wherein said agent causes the expression of a normal version of said  
RapR6 gene in cells of said cancer.

66. The method of claim 64, wherein said agent comprises a RapR6 protein or a  
35 therapeutically equivalent fragment thereof.

67. A method for treating a mammal having a cancer, comprising administering to  
said mammal a therapeutically sufficient amount of an agent, said agent regulating the  
expression of a RapR6 gene and/or activity of a protein encoded by said RapR6 gene such

that rapamycin resistance is regulated, wherein said mammal is subject to a therapy  
5 comprising administering to said mammal a therapeutically sufficient amount of rapamycin  
or an analog or derivative of rapamycin.

68. A method for treating a mammal having a cancer, comprising administering to  
said mammal i) a therapeutically sufficient amount of an agent, said agent regulating the  
10 expression of a RapR6 gene and/or activity of a protein encoded by said RapR6 gene such  
that rapamycin resistance is regulated, and ii) a therapeutically sufficient amount of  
rapamycin or an analog or derivative of rapamycin.

69. The method of claim 67 or 68, wherein said agent causes the expression of a  
normal version of said RapR6 gene in cells of said cancer.  
15

70. The method of claim 67 or 68, wherein said agent comprises a RapR6 protein or  
a therapeutically equivalent fragment thereof.

71. A method for diagnosing a cancer or a predisposition to said cancer in a  
mammal, said cancer being a result of defective regulation of a RapR6 gene, said method  
20 comprising determining an expression level of said RapR6 gene in cells of said mammal,  
wherein said expression level deviated from a predetermined threshold level indicates that  
said mammal has or is predisposed of said cancer.

72. The method of claim 71, wherein said expression level of said RapR6 gene is  
25 determined by a method comprising measuring the expression level of said RapR6 gene  
using one or more polynucleotide probes, each of said one or more polynucleotide probes  
comprising a nucleotide sequence in said RapR6 gene.

73. The method of claim 72, wherein said one or more polynucleotide probes  
30 comprise at least one polynucleotide probe comprising a nucleotide sequence within one of  
exons 1-6 of said RapR6 gene.

74. The method of claim 72, wherein said one or more polynucleotide probes  
comprise at least one polynucleotide probe comprising a nucleotide sequence of said RapR6  
gene which does not encode a WD40 domain of the encoded RapR6 protein.  
35

75. The method of claim 72, wherein said one or more polynucleotide probes  
comprise at least one polynucleotide probe comprising a nucleotide sequence within an  
intron of said RapR6 gene.

76. The method of claim 72, wherein said one or more polynucleotide probes  
5 comprise at least one polynucleotide probe comprising a nucleotide sequence comprised in the nucleotide sequence encoding a WD40 domain in the encoded RapR6 protein.

77. The method of claim 72, wherein said one or more polynucleotide probes  
comprise at least one polynucleotide probe comprising a nucleotide sequence comprised in  
10 the nucleotide sequence encoding a transmembrane domain in the encoded RapR6 protein.

78. The method of any one of claim 71-76, wherein said one or more polynucleotide probes are polynucleotide probes on a microarray.

79. A method for diagnosing a cancer or a predisposition to said cancer in a  
15 mammal, said cancer being a result of defective regulation of a RapR6 gene, said method comprising determining a level of abundance of a protein encoded by said RapR6 gene in cells of said mammal, wherein said level of abundance of said protein deviated from a predetermined threshold level indicates that said mammal has or is predisposed of said cancer.

20 80. A method for diagnosing a cancer or a predisposition to said cancer in a mammal, said cancer being a result of defective regulation of a RapR6 gene, said method comprising determining a level of activity of a protein encoded by said RapR6 gene in cells of said mammal, wherein said activity level deviated from a predetermined threshold level  
25 indicates that said mammal has or is predisposed of said cancer.

81. The method of claim 79 or 80, wherein said mammal is a human.

82. The method of claim 81, wherein said protein is a human RapR6 protein as depicted in SEQ ID NO:11.

30 83. The method of claim 83 or 84, wherein said mammal is a mouse.

84. The method of claim 83, wherein said protein is murine RapR6 protein as depicted in SEQ ID NO:3.

35 85. A method for evaluating rapamycin resistance in a cell, said method comprising determining an expression level of a RapR6 gene in said cell, wherein said expression level deviated from a predetermined threshold level indicates that said cell is rapamycin resistant.



86. The method of claim 85, wherein said expression level of said RapR6 gene is  
5 determined by a method comprising measuring the expression level of said RapR6 gene  
using one or more polynucleotide probes, each of said one or more polynucleotide probes  
comprising a nucleotide sequence in said RapR6 gene.

87. The method of claim 86, wherein said one or more polynucleotide probes  
10 comprise at least one polynucleotide probe comprising a nucleotide sequence within one of  
exons 1-6 of said RapR6 gene.

88. The method of claim 87, wherein said one or more polynucleotide probes  
comprise at least one polynucleotide probe comprising a nucleotide sequence of said RapR6  
15 gene which does not encode a WD40 domain of the encoded RapR6 protein.

89. The method of claim 87, wherein said one or more polynucleotide probes  
comprise at least one polynucleotide probe comprising a nucleotide sequence of said RapR6  
gene which does not encode a transmembrane domain of the encoded RapR6 protein.

90. The method of claim 80, wherein said one or more polynucleotide probes  
20 comprise at least one polynucleotide probe comprising a nucleotide sequence within an  
intron of said RapR6 gene.

91. The method of any one of claims 79-90, wherein said one or more  
polynucleotide probes are polynucleotide probes on a microarray.

92. The method of claim 91, wherein said one or more polynucleotide probes  
25 comprise at least one polynucleotide probe comprising a nucleotide sequence comprised in  
the nucleotide sequence encoding a WD40 domain in a RapR6 protein.

93. The method of claim 91, wherein said one or more polynucleotide probes  
30 comprise at least one polynucleotide probe comprising a nucleotide sequence comprised in  
the nucleotide sequence encoding a transmembrane domain in a RapR6 protein.

94. A method for evaluating rapamycin resistance in a cell, said method comprising  
determining a level of abundance of a protein encoded by a RapR6 gene in said cell,  
35 wherein said level of abundance of said protein deviated from a predetermined threshold  
level indicates that said cell is rapamycin resistant.

95. A method for evaluating rapamycin resistance in a cell, said method comprising  
determining a level of activity of a protein encoded by a RapR6 gene in said cell, wherein

said activity level deviated from a predetermined threshold level indicates that said cell is  
5 rapamycin resistant.

96. The method of claim 94 or 95, wherein said cell is a human cell.

97. The method of claim 96, wherein said protein is a human RapR6 protein as  
depicted in SEQ ID NO:11.  
10

98. The method of claims 94 or 95, wherein said cell is a murine cell.

99. The method of claim 98, wherein said protein is murine RapR6 protein as  
depicted in SEQ ID NO:3.

15 } 100. A method for regulating rapamycin resistance in a cell, comprising contacting  
said cell with a sufficient amount of an agent such that rapamycin resistance is regulated,  
said agent regulating the expression of a RapR6 gene and/or the activity of a protein  
encoded by said RapR6 gene.

20 101. A method for regulating rapamycin resistance in a mammal, comprising  
administering to said mammal a therapeutically sufficient amount of an agent such that  
rapamycin resistance is regulated, said agent regulating the expression of a RapR6 gene  
and/or the activity of a protein encoded by said RapR6 gene.

25 102. A method for regulating growth of a cell, comprising contacting said cell with  
i) a sufficient amount of an agent such that rapamycin resistance is regulated, said agent  
regulating the expression of a RapR6 gene and/or the activity of a protein encoded by said  
RapR6 gene; and ii) a sufficient amount of rapamycin or an analog or derivative of  
rapamycin.

30 103. The method of claims 100, 101 or 102, wherein said agent causes the  
expression of a normal version of said RapR6 gene in said cell.

104. The method of claims 100, 101 or 102, wherein said agent comprises a RapR6  
protein or a therapeutically equivalent fragment thereof.

35 105. A method of identifying an agent that is capable of regulating rapamycin  
resistance, wherein said agent is capable of modulating the expression of a RapR6 gene  
and/or the activity of a protein encoded by said RapR6 gene, said method comprising  
comparing inhibitory effect of rapamycin on cells expressing said RapR6 gene in the

presence of said agent with inhibitory effect of rapamycin on cells expressing said RapR6  
5 gene in the absence of said agent, wherein a difference in said inhibitory effect of  
rapamycin identifies said agent as capable of regulating rapamycin resistance.

106. A method of identifying an agent that is capable of regulating rapamycin  
resistance, wherein said agent is capable of modulating the expression of a RapR6 gene  
10 and/or activity of a protein encoded by said RapR6 gene, said method comprising:

(a) contacting a first cell expressing said RapR6 gene with rapamycin in the  
presence of said agent and measuring a first growth inhibitory effect;

(b) contacting a second cell expressing said RapR6 gene with rapamycin in the  
15 absence of said agent and measuring a second growth inhibitory effect; and

(c) comparing said first and second inhibitory effects measured in said step (a) and  
(b),

wherein a difference between said first and second inhibitory effects identifies said  
20 agent as capable of regulating rapamycin resistance.

107. The method of claims 105 or 106, wherein said agent comprises a RapR6  
protein or a functionally equivalent fragment thereof.

108. The method of claims 105 or 106, wherein said agent causes the expression of  
25 a normal version of said RapR6 gene in a cell.

109. A method of producing an antibody that binds specifically to a RapR6 protein,  
comprising raising said antibody against said RapR6 protein or a polypeptide comprising an  
fragment of said RapR6 protein.

30 110. The method of claim 109, wherein said RapR6 protein is a human RapR6  
protein.

111. The method of claim 109, wherein said RapR6 protein is a murine RapR6  
protein.

35 112. An antibody that binds specifically to a RapR6 protein or a fragment of said  
RapR6 protein such that binding of said antibody to said RapR6 protein regulates  
rapamycin resistance.

113. The antibody of claim 112, wherein said RapR6 protein is a human RapR6  
5 protein.

114. The antibody of claim 112, wherein said RapR6 protein is a murine RapR6  
protein.

115. An agent that regulates the expression of a RapR6 gene such that rapamycin  
10 resistance is regulated.

116. The agent of claim 115, wherein said agent comprises a molecule which  
regulates expression of said RapR6 gene.

117. The agent of claim 115, wherein said agent causes the expression of a normal  
15 version of said RapR6 gene in a cell.

118. A cell comprising a knockout DNA construct at a RapR6 locus, said knockout  
DNA construct comprising (i) a regulated promoter and (ii) a selection marker coding  
sequence under the control of said regulated promoter, wherein said regulated promoter,  
20 when activated, initiates RNA transcription to produce an RNA.

119. The cell of claim 118, wherein said knockout DNA construct is inserted in the  
intron between exon 1 and exon 2 to produce a truncated fragment of the protein encoded  
by the sequence at said RapR6 locus.

25 120. The cell of claim 118, further comprising a DNA construct encoding a  
transactivator, said DNA construct comprising (i) a promoter and (ii) a nucleotide sequence  
encoding said transactivator, said nucleotide sequence being under the control of said  
promoter, wherein said transactivator activates said regulated promoter.

30 121. The cell of claim 118, wherein said knockout DNA construct further comprises  
a rapid cloning element comprising a replication origin sequence comprising sequences for  
initiation of replication and segregation and a bacterial selection marker.

122. The cell of claim 121, wherein said replication origin sequence is an Ori and  
said bacterial selection marker is a chloramphenicol resistance gene.  
35

123. The cell of claim 122, wherein said regulated promoter is a tetracycline  
regulated promoter, and wherein said transactivator activates said regulated promoter in the  
absence of tetracycline.

124. The cell of any one of claims 118-123, wherein said cell is a rapamycin  
5 sensitive cell.

125. The cell of claim 124, wherein said cell is a human cell.

126. The cell of claim 124, wherein said cell is a murine cell.

10 127. The cell of claim 126, wherein said cell is a murine neuroblastoma N2a cell.

128. The cell of claim 127, wherein said integration site is in the intron between  
exon 1 and exon 2 of said RapR6 locus.

129. The cell of any one of claims 118-123, wherein said cell is a rapamycin  
15 resistant cell.

130. A microarray for diagnosing rapamycin resistance, said microarray comprising  
one or more polynucleotide probes, wherein each said polynucleotide probe comprises a  
nucleotide sequence in a RapR6 gene.

20 131. The microarray of claim 130, wherein said one or more polynucleotide probes  
comprise at least one polynucleotide probe comprising a nucleotide sequence within one of  
exons 1-6 of said RapR6 gene.

132. The microarray of claim 130, wherein said one or more polynucleotide probes  
25 comprise at least one polynucleotide probe comprising a nucleotide sequence within an  
intron of said RapR6 gene.

133. A kit for diagnosis of rapamycin resistance, comprising in one or more  
containers one or more polynucleotide probes, wherein each said polynucleotide probe  
30 comprises a nucleotide sequence in a RapR6 gene.

134. A kit for screening for agents which regulate rapamycin resistance and/or  
tumorigenesis, comprising in one or more containers (i) the cell of claim 118; (ii)  
tetracycline or a derivative or analog thereof; and (iii) rapamycin or a derivative or analog  
thereof.  
35